Influence of in ovo Inoculation of Probiotic Strains on the Jejunal Goblet Cell Counts and Morphometry in Peri- and Post-hatching Chicks

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Abstract
The objective of the present study was to evaluate the effects of in ovo inoculation of different probiotic strains (Bacillus subtilis, Enterococcus faecium, and Pediococcus acidilactici) on jejunal goblet cells counts and morphometry in chicken. Probiotics were inoculated into the amniotic fluid of 480 eggs (day 17 of incubation), with four treatments and five replicates. At days 21 of incubation and 3 post-hatch, counts of goblet cells were 30% and 16% higher in the jejunum of group inoculated Bacillus subtilis as compared with the control group, respectively. Inoculation of Enterococcus faecium, and Pediococcus acidilactici had no effect (P>0.05) on goblet cells counts. Inoculation of Bacillus subtilis and Pediococcus acidilactici resulted in an increase of villus height, a decrease in crypt depth and a decrease in ratio of villus height to crypt depth compared with the control group (P>0.05), at days 8 and 28 of age. As a conclusion, various effects of different probiotic strains on goblet cells count and intestinal morphometry were observed. Among probiotic strains evaluated in this study, Bacillus subtilis has higher benefit effect on goblet cells counts in the early of life and morphometry of jejunum.

Keywords: Incubation, Intestine, Morphology, Goblet cell, Probiotic

INTRODUCTION
In the modern poultry production, the contact between newborn chicks and hens is excluded, and colonization of bacteria in the gut depends on the type of bacteria present in the hatchery environment [1]. This condition exposes chicks to pathogenic bacteria colonization in the gut and causes a delay in desirable bacteria colonization [2]. The first contact of chicks with hatchery environment may include pathogen bacteria and leave gut colonization...
open to them, so it is necessary to inoculate desirable bacteria named probiotic. Different routes of early delivery of probiotics were examined, for example in ovo inoculation, immersion of eggs in probiotic medium, oral gavage, vent lip, and spraying of chick with probiotic solution [3-6]. In the previous studies that examined in ovo administration, probiotic solutions were injected into the air cell of eggs. This route of inoculation results in low hatchability. Chicken embryo swallow amniotic fluid during the last period of incubation; therefore, intra-amniotic inoculation of probiotic strains enables chicks to received desirable bacteria in the early of life without affecting the hatchability rate. Moreover, to our knowledge, in the literature the effect of intra-amniotic inoculation of different probiotic strains on intestinal characteristics of chicks was not evaluated. Therefore, the main objective of this study was to evaluate the effects of in ovo inoculation of different probiotic strains on the goblet cells counts and jejunal morphometry of broiler chicken at the peri- and post-hatch periods.

MATERIAL and METHODS

Chicks used in this study received human care based on criteria outlined in the Guide for the Care and Use of Laboratory Animals [7], and the experimental protocol was approved by the Research Committee of Islamic Azad University, Science and Research Branch (Approval date: 05.05.2013; No: 23874).

Fertile Cobb chicken eggs were obtained from a commercial hatchery from a flock with the 38 weeks of age. Eggs were incubated in a single-stage setter under the same condition of 37.6°C and 60% relative humidity and turned once per h. On day 17 of incubation, eggs with live embryo (No: 480, average weight of 58±1.1 g) were selected and weighed. In a completely randomized design, eggs were assigned to four experimental groups and five replicates of twenty four eggs per each. The four treatment groups that received in ovo 0.5 mL of sterile distilled water or probiotic mediums (10⁷ cfu) into the amniotic fluid were: 1) sterile distilled water as control group, 2) Bacillus subtilis, 3) Enterococcus faecium and 4) Pediococcus acidilactici. The in ovo inoculation procedure was performed as described by Tako et al. [8]. Solution was inoculated with a suitable needle inserted into the amniotic fluid, which was identified by candling. After inoculation, the hole in egg wall was sealed with cellophane tape, and eggs were placed in hatching trays. Upon hatching the chickens were allocated to related floor pens and raised for 6 weeks. Chickens management (water, feed, light program and pen environment) were based on Cobb 500 broiler chickens [9].

On days of 19 and 21 peri-hatch and days 1, 3, 8 and 28 post-hatch, two birds per each replicate were randomly selected, anesthetized with diethyl ether and caecal removed. The entry of caecal was sealed, removed and placed in ice and used for microbial assays. Also, samples of jejenum (3 cm) were taken and placed in buffered formalin solution (10%) for intestinal morphometry and goblet cells count. Histo-preparation was done according to the method described by Ljll et al. [10]. Goblet cell count was determined by double-stained of samples with Periodic Acid-Schiff and hematoxylin according to the method of Horn et al. [11]. The goblet cells were counted in scale of 300 µm of epithelium length.

The normality of data was evaluated using Kolmogorov-Smirnov test. Then data were analyzed using the GLM procedure of SAS for Windows, version 9.1 (SAS Institute Inc., Cary, NC). Means were separated using Duncan's Multiple Comparison test (P<0.05).

RESULTS

There was a difference (P<0.05) between chicks received Bacillus subtilis and other treatments for goblet cells counts on day 21 of incubation and day 3 post-hatch (Table 1). Differences among treatment for goblet cells count on day 19 peri-hatch and days 8 and 28 post-hatch were not significant statistically (P>0.05).

The means of jujunal villus height, crypt depth, and villus height: Crypt depth ratio are presented in Table 2. There were no differences (P>0.05) among treatment for mentioned traits on days 1 and 3 post-hatch, but on days 8 and 28 post-hatch differences were appeared among treatment (P<0.05). Inoculation of Bacillus subtilis and Pediococcus acidilactici resulted in increase of villus height and decrease in crypt depth and their ratio compared with the control group (P<0.05). There were no differences (P>0.05) for these traits between Enterococcus faecium and the control group.

DISCUSSION

Inoculation of probiotic bacteria via oral feeding is now recognized as a suitable route to reduce the risk of
intestinal infection by pathogenic bacteria [2]. An interesting study demonstrated that the time of initial intestinal colonization by desirable bacteria play an important role on the colonization of pathogens [1]. In the previous studies [5,6,8,12], the protection effects of in ovo inoculation or other route administration of probiotics against Salmonella infection were investigated, but the effects of inoculation of different probiotic strains on intestinal morphometry, and goblet cells count have not been attended. The main objective of this study was to evaluate the effect of three probiotic strains, *Bacillus subtilis*, *Pediococcus acidilactici* and *Enterococcus faecium*, on intestinal characteristics.

Chicks received *Bacillus subtilis* had higher goblet cells counts than the control group and those received other probiotic strains. An interesting study [13] showed that dietary factors and microbiota could affect goblet cell numbers. Feeding probiotic to the turkey pouls has been reported to increase the goblet cell number in the small intestine, which can protect epithelia from pathogenic bacteria [14]. Mucin production is correlated with the goblet cells number and if a pathogen enters via the digestive tract, a thick mucus layer produced by goblet cells, will block the pathogen from penetrating the host’s cells.

There were no differences among treatment for mentioned traits on days 1 and 3 post-hatch. In agreement to our finding, Santin et al.[15] with feeding *Saccharomyces cerevisiae* and Sieo et al.[16] with six Lactobacillus strains reported no differences in the small intestine morphometry. Probiotics strains were inoculated at day 17 of incubation and it seems that probiotics needs more times for research funding support. We also thank all staffs in the poultry unit, for the assistance in the care and feeding of chicks used in this research.

In inoculation of *Bacillus subtilis* and *Pediococcus acidilactici* resulted in the increase of villus height and decrease in crypt depth and their ratio compared with control group on days 8 and 28 post-hatch. The increase in villus height due to the probiotic inoculation could be considered important and beneficial for the absorptive capacity of jejunum. An increase in the villus height suggests increase in the surface area capable of higher absorption of nutrients. *Enterococcus faecium* had no effect on intestinal morphometry parameters. In contrast, Chichłowski et al.[17] and Samli et al.[18] reported that inclusion of *Enterococcus faecium* increased the jejunal villus height and decreased the villus crypt depth as compared with the control group.

As a conclusion, various effects of different probiotic strains on goblet cells count and intestinal morphometry were observed. Among probiotic strains evaluated in this study, *Bacillus subtilis* has higher benefit effect on goblet cells counts in the early of life and morphometry of jejunum.

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### References


